RESEARCH ARTICLE

Amplification of Aqueous Solubility of Progesterone Using Melt-Granulation Technique

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ABSTRACT

The objective of the present research was to enhance the rate of dissolution of progesterone by boosting the hormone's solubility in water. This research determines whether or not employing melt granulation techniques with various polymers may improve the degree to which progesterone is soluble. When looking into the interactions between drug carriers and other substances, researchers turned to techniques such as X-ray diffraction (XRD), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR). PEG 6000 (1:1.5) demonstrated the highest solubility, followed by PEG 6000 (1:1) > Gelucire 50/13 (1:1.5) > Gelucire 50/13 (1:1). Increasing the solubility of the weakly soluble progesterone was demonstrated by these findings. Melt granulation on polymers boosted progesterone's dissolution rate, which in turn can raise oral bioavailability.

Keywords: Progesterone, Bioavailability, Gelucire, PEG 6000, solubility.

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INTRODUCTION

Medicinal particle engineering is the study of creating particles with the ideal size, size distribution, shape, density, surface chemistry, internal structure, and/or powder characteristics to optimize the efficacy of active pharmaceutical components.[1-3] One of the most common and significant issues now faced by formulation scientists in the pharmaceutical business is the development of oral administration formulations for drugs with low solubility.[4-6] Almost 40% of the new medication candidates are found to have solubility issues.[7-8] Subpar water solubility correlates to slow release and poor absorption.[9-10] Since a high dose is needed to have the desired effect, the medicine may become hazardous if given in excess.[11]

Since progesterone isn't very soluble in water, this study aimed to determine whether or not the melt granulation process may be used to make the medicine more palatable to take orally.[12,13] It's being used as a case in point because it's a medicine that doesn't dissolve in water.[14] Progesterone's solubility and dissolution rate were improved through conjugation with several carriers.[15] Granules were formed using the melt granulation method and physical mixing. Increasing progesterone's water solubility aimed to increase the hormone's bioavailability after oral delivery. This study aimed to improve the solubility of progesterone by applying melt granulation techniques with various polymers.

MATERIALS AND METHODS

Materials

Sun Pharma Pvt. Ltd. of Ahmadabad generously donated progesterone for this study. The chemicals poloxamer 188, polyethylene glycol 6000, and gelucire 50/13 were all purchased from Sigma-Aldrich. All reagents and compounds employed were of analytical quality and required no further purification before use. In the course of the experiments, double-distilled water was employed.

Preparation of the Physical Mixture

To compare with the optimized formulation, the drug and poloxamer 188 at the same amount were mixed to get the physical mixture. After being mixed in a mortar, the granules were sized using a #40 sieve. The resulting mixture was stored in a desiccator.

UV analysis

Progesterone (1-mg/10 mL) was dissolved in water to create a stock solution, which was then further diluted to yield a range of concentrations from 2 to 18 g/mL. With a UV-visible spectrophotometer with water serving as a blank, we measured the absorbance of the solutions at 241 nm and plotted the results against a standard curve to get a sense of how the concentration affected the absorbance.

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Melt granulation method

About 250 mg of poloxamer 188 was taken in a beaker and was melted at its melting point, followed by the addition of 500 mg of the drug with continuous stirring using a magnetic stirrer to get a concentration of 1:0.5. Similarly, about 500 mg of poloxamer 188 was taken in a beaker and was melted at its melting point followed by addition of 500 mg of the drug with continuous stirring using a magnetic stirrer to get a concentration of 1:1. Further about 750 mg of poloxamer 188 was taken in a beaker and was melted at its melting point followed by addition of 500 mg of the drug with continuous stirring using a magnetic stirrer to get a concentration of 1:1.5. The same procedure was repeated for PEG 6000 and Gelucire 50/13. For optimal mechanical strength in agglomerates generated through melt pelletization and melt granulation processes, it is advantageous for the meltable materials to possess both elevated viscosity and reduced particle size. Based on the results; poloxamer 188 was optimized for further formulations with better results.

Fourier transform infrared analysis

The IR Spectrophotometer was used to perform this study. Around 2 mg samples were scanned between 4000 and 500 cm^{-1} using a dry potassium bromide mixture.

X-ray diffractometer *analysis*

The X-ray diffractometer (XRD) performed the XRD of pure drug, polymer, physical mixture, and the optimized formulation. The samples were heated from 10 to 80°C at a scanning rate of 2°C/min.[2]

DSC analysis

Progesterone, the physical mixture, and the best melt granulation batch differential scanning calorimetry (DSC) (Mettler Toledo) thermograms were taken in DSC over a temperature range of 40 to 360°C at a flow rate of 30 mL/min of nitrogen.

Experimental Design

The impact of factors on melt granulates' stability and integrity was initially screened for in a series of preliminary investigations.[5] The concentration of poloxamer 188 was shown to be a critical formulation parameter, while stirrer speed was found to be a critical process parameter. Using a 3^2-factorial design, we systematically tested the effects of varying three levels of key process and formulation parameters (-1, 0, and +1) on the critical quality attributes of the optimized batch. In these experiments, the amount of drug and stirring time were kept constant throughout the trial.

Solubility study of melt granulated formulation

Sample equivalent to 25 mg of drug concentration was taken in separate volumetric flasks of all the aforesaid concentrations in 10 mL water, kept for shaking for approximately 3 hours, and then diluted to obtain the absorbance reading in beer lamberts range. The data was collected in triplicates (n = 3).

Dissolution studies

The rate of dissolution of progesterone from melt granules was assessed *in-vitro* and compared to a commercially available formulation (Progesterone Capsule) using the basket method. The experiment was conducted in a 900 mL vessel at 37 ± 0.5°C, constant-speed 75 rpm, in 0.1 N HCl. At regular intervals over one hour, 5 mL aliquots of the test sample were manually collected. The withdrawn samples were substituted with fresh media of equal volume at the same temperature. Retrieved solutions were appropriately diluted and subjected to progesterone content testing. The quantity of dissolved progesterone was measured at 241 nm using a UV spectrophotometer and calculated to determine the percentage of cumulative drug release.

Table 1: Standard UV curve of progesterone

Final conc. (µg/mL)	*Absorbance*
2.00	0.136
4.00	0.233
6.00	0.367
8.00	0.469
10.00	0.569
12.00	0.687
14.00	0.782
16.00	0.89
18.00	0.988
Slope	0.054
Intercept	0.033

RESULTS AND DISCUSSION

UV Analysis

The standard calibration curve was plotted between the 2 to 18 µg/mL concentration range as presented in Table 1 and Figure 1.

FTIR analysis

The FTIR spectra (Figures 2 & 3) were captured using an IR spectrophotometer (Alpha T Bruker). Around 2 mg of the sample was scanned between 4000 and 500 cm^{-1} using a dry potassium bromide mixture.

FTIR spectra of free-form progesterone and its formulation are shown in Figures 1 and 2, respectively. The drug's spectra showed the signature peak at 2924 cm^{-1}, which is indicative of C-H stretching in alkanes. Its sharp peak can characterize the carbonyl stretch (C=O) in medicine at 1738 cm^{-1}, whereas the peak at 1660 cm^{-1} denotes the C=C stretch. Peaks at 1438 cm^{-1} suggest aromatic C=C stretch. Shifts in the peaks' locations, albeit slight, were noticed. The spectra of the physical combination and the improved formulation showed very similar absorbance patterns, indicating that the drug and polymers are compatible with one another. Because of this, no functional group movement has been noticed, and very minimal interactions between medication and polymers have been noted.

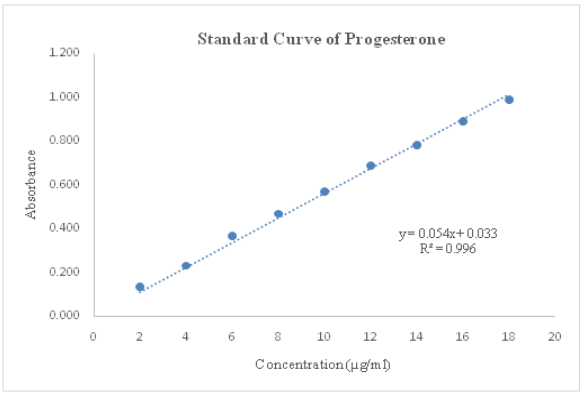

Figure 1: Standard UV curve of progesterone

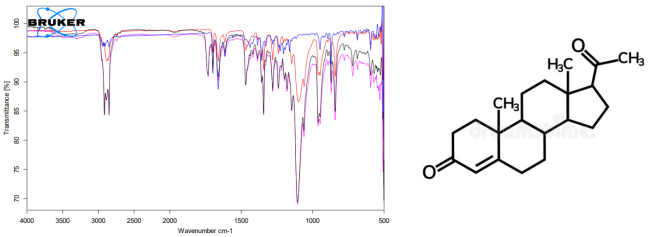

Figure 2: Overlay of FTIR of progesterone, PEG 6000, physical mixture and optimized formulation

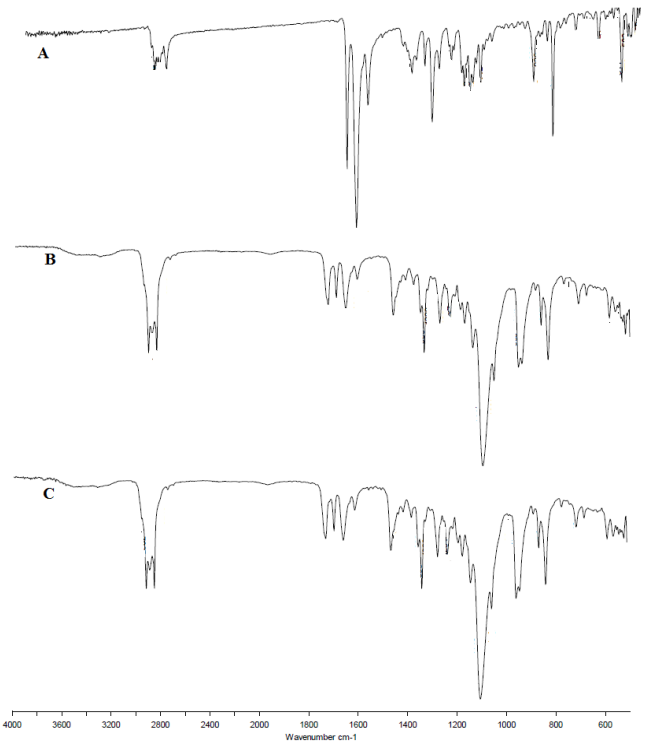

Figure 3: FTIR spectra A) Progesterone, B) Physical Mixture & C) Optimized formulation

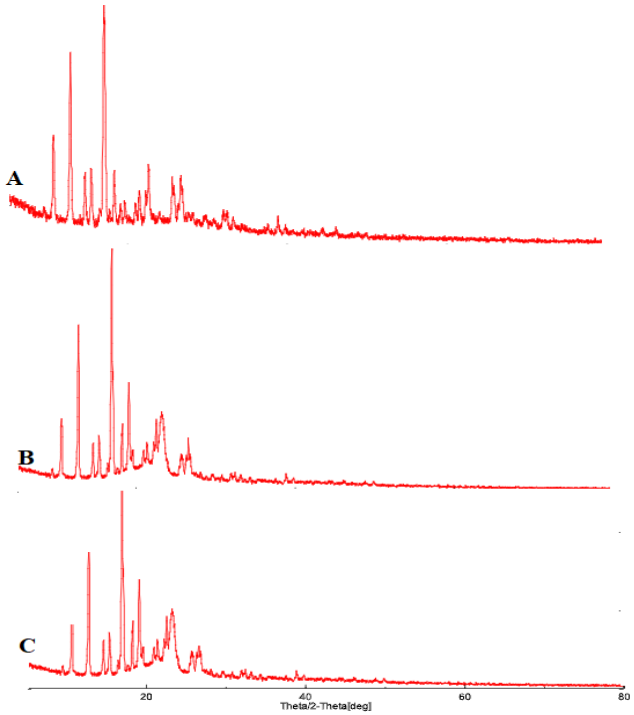

Figure 4: PXRD pattern of A) Progesterone, B) Physical Mixture & C) Optimized formulation

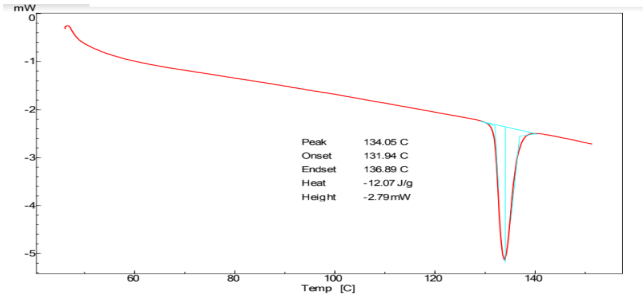

Figure 5: DSC of progesterone

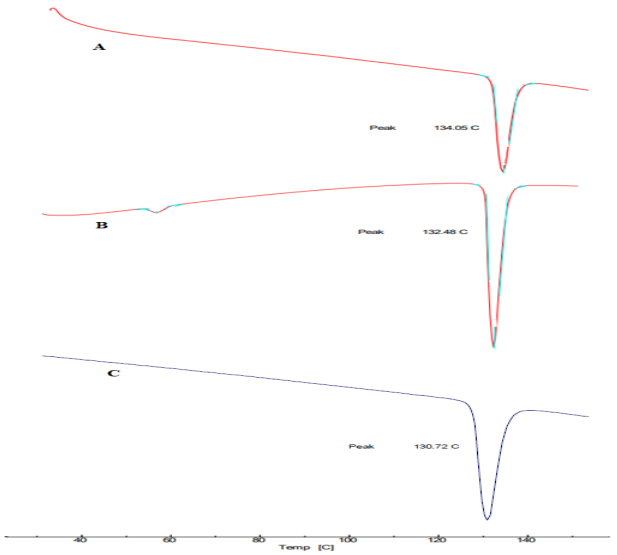

Figure 6: DSC overlay of A) Progesterone, B) Physical Mixture & C) Optimized formulation

Table 2: Preparation of melt granulates with 3^2 factorial designs

Code	*Independent variables*				*Dependent variables*	
	Formulation variables				Y_1	Y_2
	X_1	*Concentration of Poloxamer 188 (mg)*	X_2	*Stirring speed (rpm)*	*Solubility (mg/mL)*	*DE60 (%)*
MT1	−1	250	+1	3000	0.072 ± 0.004	15.63 ± 1.2
MT2	0	500	+1	3000	0.078 ± 0.003	18.31 ± 2.5
MT3	+1	750	+1	3000	0.086 ± 0.005	21.10 ± 1.7
MT4	−1	250	0	2000	0.068 ± 0.007	14.78 ± 1.4
MT5	0	500	0	2000	0.070 ± 0.002	15.31 ± 2.1
MT6	+1	750	0	2000	0.067 ± 0.006	15.22 ± 1.5
MT7	−1	250	-1	1000	0.035 ± 0.002	11.13 ± 1.2
MT8	0	500	-1	1000	0.052 ± 0.004	14.31 ± 2.7

All values are expressed as mean SD (n = 3), with +1 representing a higher level, -1 a lower level, and 0 representing the median.

Table 3: Statistical analysis

Responses	*Sources*		
	Model p-value	*Adj-R^2*	*Lack of fit test p-value*
Solubility (mg/mL)	0.0023	0.9245	0.3791
DE60 (%)	0.0033	0.9562	0.7746

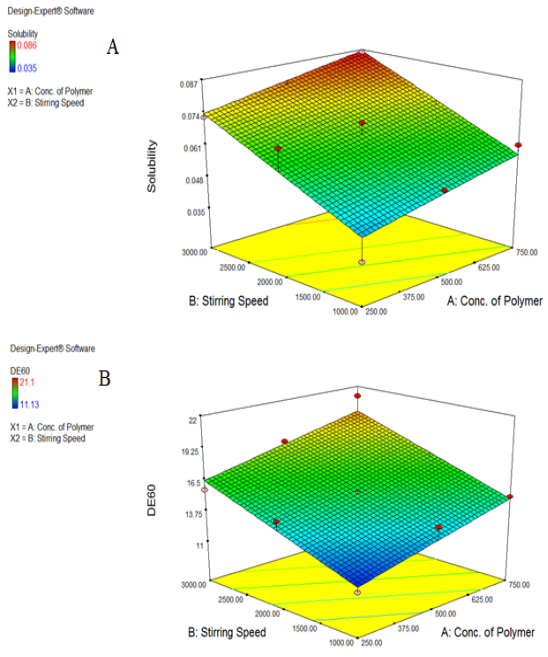

Figure 7: 3D surface response plots indicating the effect of conc. of poloxamer 188 (mg) and stirring speed (rpm) on (A) Solubility and (B) DE_{60}

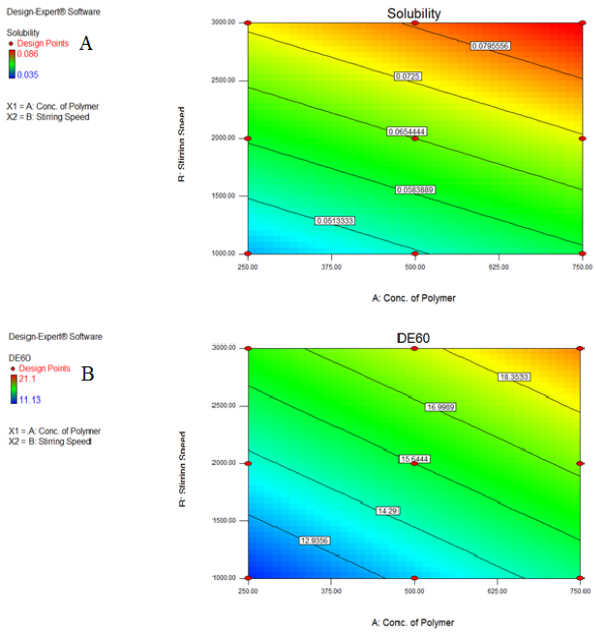

Figure 8: Contour plots showing the effect of conc. of poloxamer 188 (mg) and stirring speed (rpm) on (A) Solubility and (B) DE_{60}

XRD Analysis

To analyze the physical nature of the medication that was encapsulated, the PXRD method was employed.[9] Pure drug showed intense crystalline peaks in XRD patterns at 2θ values (Figure 4). The pattern of the physical mixture and optimized formulation revealed very distinct, intense drug peaks, indicating that the drug's crystalline properties were preserved. No dilution of the medication by the polymer network or qualitative alteration was visible in the diffractogram.

Table 4: Assessment of observed and predicted values

Factors		*Predicted value*		*Observed value**	
Conc. of Poloxamer 188 (mg)	Stirring speed (rpm)	Solubility (mg/mL)	DE60 (%)	Solubility (mg/mL)	DE_{60} (%)
750	3000	0.088	22.50	0.086 ± 0.005	21.10 ± 3.1

All values are mean ± SD (n = 3).

Table 5: Details of solubility studies by melt granulation technique

Trial no	*Sample ID*	*Absorbance value*	*Dilution factor (DF)*	*Slope*	*Con= Abs/ slope*DF(µg/ml)*	*Con in mg/ml*
Trial 1	PG:Poloxamer (1:0.5)	0.310	15	0.054	86.11	0.086
Trial 2	PG:Poloxamer (1:0.5)	0.258			71.67	0.072
Trial 1	PG:Poloxamer (1:1)	0.282			78.33	0.078
Trial 2	PG:Poloxamer (1:1)	0.244			67.78	0.068
Trial 1	PG:Poloxamer (1:1.5)	0.252			70.00	0.070
Trial 2	PG:Poloxamer (1:1.5)	0.242			67.22	0.067
Trial 1	PG:PEG6000(1:0.5)	0.125			34.72	0.035
Trial 2	PG:PEG6000(1:0.5)	0.187			51.94	0.052
Trial 1	PG:PEG6000(1:1)	0.218			60.56	0.061
Trial 2	PG:PEG6000(1:1)	0.249			69.17	0.069
Trial 1	PG:PEG6000(1:1.5)	0.222			61.67	0.062
Trial 2	PG:PEG6000(1:1.5)	0.237			65.83	0.066
Trial 1	PG:Gelucire 50/13(1:0.5)	0.781	20		216.94	0.217
Trial 2	PG:Gelucire 50/13(1:0.5)	0.654			181.67	0.182
Trial 1	PG:Gelucire 50/13(1:1)	0.474			175.56	0.176
Trial 2	PG:Gelucire 50/13(1:1)	0.685			253.70	0.254
Trial 1	PG:Gelucire 50/13(1:1.5)	0.657			243.33	0.243
Trial 2	PG:Gelucire 50/13(1:1.5)	0.672			248.89	0.249

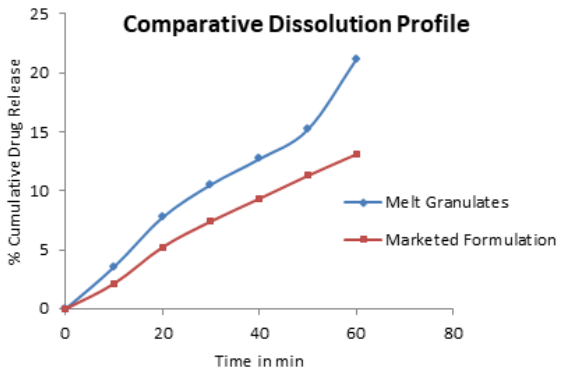

Figure 9: Dissolution profiles of optimized formulation with marketed formulation

Table 6: Comparative dissolution profile of optimized formulation of progesterone & marketed formulation

Time (minutes)	*Melt granulates of progesterone*	*Marketed formulation of progesterone*
0	0	0
10	3.54 ± 1.03	2.13 ± 1.09
20	7.73 ± 1.19	5.21 ± 0.66
30	10.51 ± 0.98	7.41 ± 0.84
40	12.69 ± 2.03	9.33 ± 0.94
50	15.17 ± 1.49	11.27 ± 1.21
60	21.10 ± 1.71	13.08 ± 1.24

Table 7: Drug release kinetics of optimised batch

Formulation	*Zero*	*First*	*Higuchi*	*Hixon-Crowell*	*Korsmeyer – Peppas*	
	R^2				R^2	*Release Exponent (n)*
Optimized batch	0.973	0.966	0.951	0.969	0.986	0.931

DSC analysis

Progesterone melts at 126 to 131°C. The thermogram of progesterone in Figure 5 exhibited a distinct melting peak at 134.05°C, indicating the molecule possessed a crystalline structure. In the tests, the physical mixture's endothermic peak and the final drug formulation's endothermic peak were found to be at 132.48 and 130.72°C, respectively (Figure 6). The outcomes shown above demonstrate that crystallinity and the primary interactions remained steady throughout the drug's formulation process.

Experimental Design

The results of the regression analysis of the experimental runs yielded equations with statistically significant F ratios ($p < 0.05$) (Tables 2 & 3).

These model equations provided a good fit to the data. There is synergy when the sign is positive and antagonism when it is negative.

Solubility (mg/mL) = $0.065 + (0.03 \times X_1) + (0.015 \times X_2)$ (Linear model) …… (1)

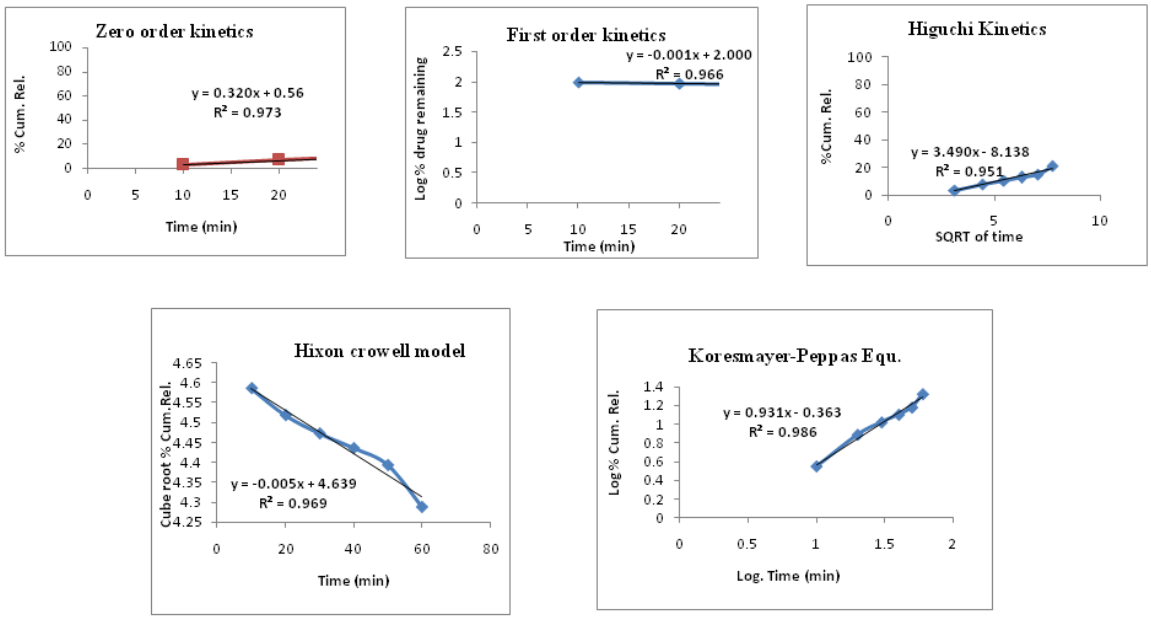

Figure 10: Drug release kinetics of optimized progesterone formulation

$$DE_{60}\ (\%) = 15.64 + (1.63 \times X_1) - (2.43 \times X_2)\ \text{(Linear model)} \quad \ldots\ldots\ldots (2)$$

Where Poloxamer 188 concentration (mg) and stirring speed (rpm) are denoted by X_1 and X_2, respectively

Solubility (mg/mL) was shown to increase with both poloxamer 188 concentration and stirring speed (Equation 1). Equation (2) showed that the concentration of Poloxamer 188 is directly related to DE_{60} (%), and the stirring rate was found to be inversely related to $_{DE60}$ (%). The results were supported by the response surface plots shown in Figures 7 and 8.

Design-Expert was used to calculate the best possible formulation by analyzing the desirability function. Table 4 displays the findings of checking the model, which include a comparison of the observed and anticipated solubility and DE60% values based on the model equations.

Solubility analysis

Progesterone solubility with different polymers indicated that PEG 6000 at a concentration of 1:1.5 was showing the highest solubility, followed by PEG 6000 (1:1) > Gelucire 50/13 (1:1.5) >Gelucire 50/13 (1:1) as shown in Table 5. Further polymeric samples were showing more or less the same solubility on the lower side.

In-vitro dissolution study

Figure 9 depicts the quicker drug release outline achieved by the optimized batch (MT3). The optimized batch's solubility profile in 0.1 N HCl showed a striking enhancement in the rate of dissolution compared to commercially available progesterone formulations. Drug release from MT3 was 21.10%, while release from a commercial capsule was just 13.1% (Table 6). The drug diffused quickly across the polymeric matrix, resulting in rapid drug release. It follows many release mechanisms other than diffusion, as indicated by the 'n' values of 0.931 in the Korsmeyer-Peppas model (Table 7). The optimized formulation MT3 followed the Korsmeyer-Peppas model for dissolution (R^2 = 0.986; see Table 7 and Figure 10) with release exponent (n) values of 0.931, indicating that drug release follows various release mechanisms in addition to diffusion.

CONCLUSION

The objective of this work was fulfilled by improving the rate of dissolution of progesterone by boosting the hormone's water solubility. The melt granulation technique was used with a variety of polymers to improve the degree of solubility of progesterone. The DSC & FTIR outcomes showed that crystallinity and the primary interactions remained steady throughout the drug's formulation process. The most recent study revealed enhanced progesterone solubility in water. Progesterone's dissolution rate was the key factor influencing increased oral bioavailability, which was improved by using the melt granulation process on polymers. Making suitable formulations with better attributes can be a cost-effective strategy.

REFERENCES

1. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water-soluble drugs. Drug discovery today. 2007 Dec 1;12(23-24):1068-75. https://doi.org/10.1016/j.drudis.2007.09.005.
2. Butreddy A, Sarabu S, Almutairi M, Ajjarapu S, Kolimi P, Bandari S, Repka MA. Hot-melt extruded hydroxypropyl methylcellulose acetate succinate based amorphous solid dispersions: Impact of polymeric combinations on supersaturation kinetics and dissolution performance. International Journal of Pharmaceutics. 2022 Mar 5;615:121471. https://doi.org/10.1016/j.ijpharm.2022.121471

3. Argade PS, Magar DD, Saudagar RB. Solid dispersion: solubility enhancement technique for poorly water-soluble drugs. Journal of Advanced Pharmacy Education & Research. 2013 Oct;3(4):427-439
4. Madan JR, Kamate VJ, Dua K, Awasthi R. Improving the solubility of nevirapine using a hydrotropy and mixed hydrotropy based solid dispersion approach. Polymers in Medicine. 2017;47(2):83-90. https://doi.org/10.17219/pim/77093.
5. Mamatha P, Bhikshapathi DVRN. Preparation and In-vitro Evaluation of Pemigatinib Nanosponges Tablets by Box-Behnken Design. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):791-800. https://doi.org/10.25258/ijpqa.14.3.56.
6. Kshirsagar S, Choudhari M, Sathyan R, Dhore S. Solubility enhancement by various techniques based on pharmaceutical and medicinal chemistry approach: an overview. Asian Journal of Pharmacy and Technology. 2019;9(2):141-6. https://doi.org/10.5958/2231-5713.2019.00024.2
7. Sahoo CK, Reddy GS, Vojjala A, Reddy BV. Bioavailability enhancement for poorly soluble drugs: A review. Innoriginal: International Journal of Sciences. 2018 Jun 6:1-6.
8. Dhillon B, Goyal NK, Malviya R, Sharma PK. Poorly water-soluble drugs: Change in solubility for improved dissolution characteristics a review. Global Journal of Pharmacology. 2014;8(1):26-35. https://doi.org/10.5829/idosi.gjp.2014.8.1.81201
9. Moinuddin, Neekhra S, Ahmad S, Swarnkar SK, Gupta D, Khunteta A, Jain P, Jain S. Evaluation of Oral Bioavailability and In-vivo Anti-leukemic Potential of Dasatinib Loaded Solid Lipid Nanoparticles. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):587-591. https://doi.org/10.25258/ijpqa.14.3.21.
10. Lopez RA, Goodman AB, Rhodes M, Blomberg JA, Heller J. The anticancer activity of the transcription inhibitor terameprocol (meso-tetra-O-methyl nordihydroguaiaretic acid) formulated for systemic administration. Anti-cancer drugs. 2007 Sep 1;18(8):933-9. https://doi.org/10.1097/CAD.0b013e32813148e0.
11. Chen X, Partheniadis I, Nikolakakis I, Al-Obaidi H. Solubility improvement of progesterone from solid dispersions prepared by solvent evaporation and co-milling. Polymers. 2020 Apr 7;12(4):854. https://doi.org/10.3390/polym12040854
12. Kannao SU, Bakade BV. Solid Dispersion–A Technique for Solubility Enhancement of Weakly Water-Soluble Drug–A Review. Indo American Journal of Pharm Research. 2014;4(6).
13. Narala S, Komanduri N, Nyavanandi D, Youssef AA, Mandati P, Alzahrani A, Kolimi P, Narala N, Repka MA. Hard gelatin capsules containing hot melt extruded solid crystal suspension of carbamazepine for improving dissolution: Preparation and in vitro evaluation. Journal of Drug Delivery Science and Technology. 2023 Apr 1;82:104384. https://doi.org/10.1016/j.jddst.2023.104384
14. Rodge PJ, Shirolkar SV. Solubility Enhancement of Itraconazole by Centrifugal Melt Spinning Technique. International Journal of Drug Delivery Technology. 2023;13(3):812-817. https://doi.org/10.25258/ijddt.13.3.06
15. Rincón-López J, Almanza-Arjona YC, Riascos AP, Rojas-Aguirre Y. Technological evolution of cyclodextrins in the pharmaceutical field. Journal of Drug Delivery Science and Technology. 2021 Feb 1; 61:102156. https://doi.org/10.1016/j.jddst.2020.102156